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DR. IVOR GRIFFITH
1891-1961

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AMERICAN JOURNAL OF PHARMACY

AND THE SCIENCES SUPPORTING PUBLIC HEALTH

Since 1825

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IN MEMORIAM

IVOR GRIFFITH

1891 - 1961

DR. IVOR GRIFFITH, President of the Philadelphia College of Pharmacy and Science since 1941, died at his home in Germantown, Philadelphia, May 16, 1961.

Although his recovery from recent surgery had been retarded because of the serious illness and death of his wife, Hilda, he had every hope of returning to his duties at the College and was anticipating presiding over the 140th Commencement Exercises in June.

But fate ruled otherwise, and suddenly his agile mind and his facile tongue were stilled, and the educational institution with which he had been affiliated for more than 50 years, and the profession of which he was so proud to be a member suffered an irreparable loss. He shall be missed for a long while to come.

Born in Wales in 1891, Dr. Griffith came to the United States with his family in 1907 to settle in Bangor, Pa., where his father, the Reverend John W. Griffith, was called as pastor of a Methodist Church.

In 1912 he received the degree of Doctor of Pharmacy from the Philadelphia College of Pharmacy, as the institution was then known, following which he was associated with the College continuously until the date of his death—as assistant, instructor, professor, dean, and then president.

He was also Honorary Professor of Organic Chemistry at the Wagner Free Institute of Science in Philadelphia, and had formerly been Research Director for the John B. Stetson Hat Company, the Frank H. Lee Hat Company, and the McNeil Laboratories.

He was a member of the American Institute of Chemists, the American Chemical Society, a past president of the American Pharmaceutical Association, and a Fellow of the Royal Society of Arts, London, England. He held membership in the Union League, the Rotary Club of Philadelphia, and the Penn Club. He was a past president of the Welsh Society and St. David's Society of Philadelphia.

In 1921 he received the degree of Master in Pharmacy from the Philadelphia College of Pharmacy and Science, and in 1930 Bucknell University granted him the honorary degree of Doctor of Science.

Dr. Griffith was well known as a horticulturist, as an educator, as an author, and as a speaker. His philosophy of teaching, so well remembered by the many hundreds of students he had taught at the College over a span of half a century is reflected in his own poem, "The Creed of the Pedagogic Rebel"—

I teach—try to reach
To my ultimate objective, not with
formulas collective,
Not with methods pedagogic, nor with
regimented logic.
I detest abused statistics, and those
lecture hall ballistics.
Let the Czars of Education
Take their concrete transportation;
I'll take those enchanting by-ways
Far removed from charted highways;
Paths of beauty through the valleys,
Paths of duty in the alleys—
I shall learn as well as teach,
I shall pray as well as preach.
And I'll make a happy landing
Sooner far than those outstanding
Exhibitionists of "Teach."
For my lads, grown men, will say:
"He taught us how to watch and pray
And live rejoicing every day."
Happy Day—Oh, Happy Day—
When teachers all shall teach that way!

He had edited two volumes of his articles, poems and lectures—"Lobscows" and "To the Lilacs." For twenty years—1921 to 1941—he had been Editor of the *American Journal of Pharmacy*.

During World War II, he originated the National Quinine Pool, through which stores of quinine salts in the United States were collected, refined, and used as an antimalarial by our troops fighting in the tropics.

He was the most recent recipient (December, 1960) of the Remington Medal of the New York Branch of the American Pharmaceutical Association. He is survived by two daughters, Mrs. Doris Schiller and Mrs. Gwenn Bunnell, and a sister, Mrs. Bessie Jones.

The following resolution adopted by the Faculty Council of the College gives expression to the reaction of his colleagues at his sudden passing—

> "Dr. Ivor Griffith, scientist, educator, and master executive is the only man in the 140 years of our Alma Mater's history who over a period of fifty years has served as student, alumnus, president of the alumni association, instructor, professor, head of the department of pharmacy, dean and president of the Philadelphia College of Pharmacy and Science. He has done yeoman service in organizing and directing the affairs of the College and he has established it on a firm foundation. His leadership, forcefulness of personality, and self-sacrificing labors have brought the College through its period of greatest growth. His has been a vigorous life, pulsing with fullness of service.
>
> "We bow to the inevitable in Dr. Griffith's passing. His memory, which we shall always hold in deep affection, shall be our pilot light. He will be forever revered, and we are the richer for his having been with us. His constructive work will be held in lasting remembrance by grateful coworkers who know the extent to which he gave of himself in his day that others may have like opportunity tomorrow."

Dr. Griffith's own outlook on death, quoted below, might well be adopted by all of us—

> Think of it as sunset—
> There is no death!
> A radiant sunset at the end
> Hushing the breath;
> With every cloud that dimmed the day
> Dispersed to vapor thin
> And Heaven itself so glad to say
> "Good morning—friend—come in!"

JOHN E. KRAMER

E D I T O R I A L

SEEDS OF DESTRUCTION

IN OUR current preoccupation and concern over the possibility that the United States might be destroyed by nuclear warfare, it is ironic that little or no attention seems to be given to that which is most likely to destroy America as we know it and our democratic way of life. There seems to be the feeling that all that need be done is for Congress to appropriate more billions for defense and that we engage in building more and more terrible machines of war with a destructive power sufficient to annihilate all living matter from the earth. Unless human beings have gone totally mad, an all-out nuclear war is unthinkable since it would destroy both victor and vanquished leaving nothing worthwhile behind. While we presume this country must be militarily strong as a means of checkmating those who might wish to destroy us, in all likelihood, our eventual destruction will not be occasioned by precipitous nuclear attack from without but by an insidious, many faceted deterioration from within. In fact, the seeds which could well grow and lead to our eventual destruction are planted all about us and every area of American life presently seems to be nurturing these seeds and encouraging their growth. Basically, the problem can be stated quite simply and it is that far too many Americans live lives strictly devoted to themselves and self interest and wherever an occasion requires a decision between what is best for America and what is best for themselves they decide for the latter. We see it, for example, in politics and government when those who are presumed to be leaders do and say what they know will be popular with the people rather than that which in the ultimate analysis would be best for the country. We see it within industry when the urge for quick and easy profits obscures the sense of public responsibility and what is best for the common good. We see it in the ranks of labor when workers fully protected by labor legislation and strong unions do a shoddy job and callously ignore the effect which this has on Americans generally. Even teachers and students, rather than dedicate themselves fully to the serious business

of sound, effective education, seem content only too often with that which will just get by rather than that which is the very best possible.

As we view those who are committed to a totally different philosophy than ourselves and who openly boast that they will eventually "bury" us, we should not be so smug in our present ease and contentment as to brush off such claims or deride those who make them. Some of these countries have made truly remarkable progress and they have done so because, willingly or not, their citizens are working for the common good and doing so to the utmost of their abilities and capacities. Such a spirit has not been in evidence in this country for a long time but it was indeed this same spirit which changed our early struggling colonies into a world power in less than a century.

It takes but little objective study to recognize that Americans by and large are not devoting themselves to the tasks which confront us and which may spell the difference between survival and disaster. Indolence, poor workmanship, and irresponsibility not only go unchecked but there seems almost to be a premium placed on them. It was indeed a sad day in America when political expediency was found to pay off while those who stood firm on the bedrock of principle went down into defeat. What this country needs today is millions of Americans who will live and work for the United States and this, we suggest, cannot be accomplished with money alone. It requires a dedication such as may be seen elsewhere in the world on the part of those who are both tough and unrelenting. Truly, the seeds of destruction which are all about us can only be denied growth by a resolute and determined America made up of men who are not just willing to die for their country but even more determined to live and work for it to the very best of their abilities.

L. F. Tice

A RAPID METHOD FOR SERUM URIC ACID WITHOUT CYANIDE

By Grace F. Grossmann,* Alfred Grossmann,* Edward Kravitz,** and Robert L. Pollack **

THE determination of uric acid concentration of blood has been the subject of numerous papers, going back to the earliest years of the present century and occupying the attentions of many investigators (1-5). These methods all use a phosphotungstic acid protein-free filtrate, which is made alkaline with cyanide or carbonate, followed by a phosphotungstic acid reagent to produce a blue color for measurement. None of these methods has been completely satisfactory. Those employing cyanide have the drawback of a dangerously toxic solution. At the same time, this solution is not stable, resulting in possible errors because of high blank values obtained with its use. The carbonate procedures have been reported to produce cloudy solutions unless the phosphotungstic acid is fortified with a lithium salt (10). In first-hand experience, even with added lithium sulfate, the writers have frequently observed cloudiness.

The method described in this paper uses sodium tungstate to provide the alkaline medium. The reagent is stable and does not require the use of lithium salts to fortify the phosphotungstic acid reagent.

An ideal method for the routine clinical laboratory should have the following attributes: (1) Good reproducibility. (2) Adherence to Beer's Law throughout the normal range and a significant portion of the abnormal range. (3) Low and consistent reagent blanks. (4) Stability of reagents and standards. (5) No interference by formation of "cloudiness." (6) Minimal toxicity. (7) Rapid and simple technique and no need for special apparatus. In the judgment of the authors, the method described in this paper appears to meet these criteria. It has been employed successfully at one of the cooperating laboratories (Newark) for about a year and at the other (Grace) for nearly four years.

* Grace F. Grossmann is Associate Director and Alfred Grossmann is Director of Grace Laboratories, 5909 Ridge Ave., Philadelphia 28, Pa.

** Dr. Kravitz is Director and Dr. Pollack is consultant, Newark Medical Laboratory, Newark, Delaware.

Materials and Methods

Reagents

1. Phosphotungstic Acid Reagent (6): Into a 1500 ml. Florence flask, place 800 ml. water, dissolve 100 Gm. sodium tungstate (Folin), add 80 ml. phosphoric acid (85%), and mix well. Reflux gently for two hours, cool to room temperature, and dilute to 1000 ml. with water. Filter if necessary. Store in brown glass bottle.

2. Concentrated Sodium Tungstate Reagent: Weigh 120 Gm. sodium tungstate (Folin) into a polyethylene bottle. Add 180 ml. water and shake until dissolved. Filter if a precipitate develops.

3. Sulfuric acid, $\frac{1}{12}$ Normal.

4. Sodium tungstate (Folin) 10% solution.

5. Benzoic acid, saturated solution in water.

6. Stock standard (7): Into a dry 1000 ml. volumetric flask, weigh 1.0 Gm. uric acid. Add 150 ml. hot lithium carbonate solution (0.4%). Stopper flask, shake vigorously under running hot tap water until all uric acid is dissolved. Cool to room temperature. Add 20 ml. 40% formaldehyde and about 500 ml. water. While shaking, add 25 ml. sulfuric acid (1.0 N). Dilute to volume, mix and preserve in a brown glass bottle away from light.

7. Dilute working standard equivalent to 5 mg. per 100 ml.: In a 200 ml. volumetric flask, add 1.0 ml. stock standard; then, dilute to mark with saturated benzoic acid solution. Preserve in a brown glass bottle away from light. Other dilutions may similarly be prepared.

Procedure

Protein free filtrate: Into an appropriate container, place 16 ml. $\frac{1}{12}$ N sulfuric acid and 2.0 ml. serum. Mix well; then, add 2.0 ml. 10% sodium tungstate and mix again. Filter. Into suitable colorimeter tubes, place the following: 5.0 ml. protein free filtrate (or appropriate standard, or water for blank). Add 2.5 ml. Concentrated Sodium Tungstate Reagent. Mix well. Add 0.5 ml. Phosphotungstic Acid Reagent. Mix well. Read at 660 mμ, after standing for five minutes.

Results and Discussion

Ten protein-free filtrates were prepared from ten batches of pooled sera. Ten uric acid determinations were performed on each of the ten filtrates using the proposed method; the results were compared to ten determinations on each of the filtrates utilizing the method of Folin (8). A series of standards with appropriate concentrations of uric acid were run and were read in the Lumetron Colorimeter at 650 mμ and with the Klett-Summerson Colorimeter at 660 mμ.

The results were tabulated, Table 1, along with pertinent statistical data. The mathematical treatment was based upon standard methods (9).

1. *Reproducibility:* The greater reproducibility of the newer method is apparent from its consistently lower standard deviation and coefficient of variation. This shows greater precision both within each test of ten replicates and among the ten tests. This superior precision is further emphasized by Figures 1 and 2. Each graph shows a

TABLE 1

SUMMARY OF STATISTICAL DATA

Test	*Mean*		*Extremes*		*Standard Deviation*		*Coefficient of Variation*	
	New	*Folin*	*New*	*Folin*	*New*	*Folin*	*New*	*Folin*
1.	4.3	4.5	4.1	3.6	0.14	0.45	3.3	10.2
			4.6	5.0				
2.	5.2	4.6	5.0	4.2	0.16	0.28	3.0	6.1
			5.5	5.2				
3.	4.5	5.1	4.5	4.3	0.07	0.39	1.6	7.7
			4.7	5.6				
4.	4.6	3.8	4.4	3.4	0.17	0.27	3.7	7.0
			4.9	4.2				
5.	3.4	3.4	3.3	3.0	0.13	0.16	3.7	4.8
			3.7	3.6				
6.	3.8	2.6	3.6	2.1	0.16	0.38	4.3	14.5
			4.1	3.2				
7.	3.5	3.2	3.4	2.8	0.07	0.26	2.0	8.1
			3.6	3.6				
8.	4.6	4.0	4.3	3.8	0.20	0.15	4.3	3.6
			4.9	4.2				
9.	3.8	3.8	3.7	3.5	0.08	0.20	2.0	5.1
			3.9	4.2				
10.	3.9	3.4	3.8	3.0	0.10	0.35	2.4	10.1
			4.0	4.1				

narrower range of variability, as well as lower values for the standard deviation and coefficient of variation, for the newer method.

2. *Beer's Law:* The graph shown in Figure 3 was plotted from typical data obtained at 650 mμ using a Lumetron Colorimeter. A similar straight line was obtained on the Klett-Summerson Colorimeter at 660 mμ.

3. *Reagent Blanks:* All reagents were kept at room temperature for a period of ten hours. The large increase in the Folin blank and its variability (42 to 100 Klett units) is sharply contrasted to the low and stable blank of the newer method (5 to 14 Klett units).

4. *Stability:* No significant deterioration has been detected in any reagent used in the method described in this paper, even when stored at room temperature for several years. Dilute standards have shown good stability at room temperature for at least several months.

5. *Cloudiness:* No cloudiness was observed in the newly presented procedure, in spite of the fact that lithium salts are not added to the phosphotungstic acid reagent.

6. *Minimal Toxicity:* The absence of cyanide from the reagents eliminates a very toxic material.

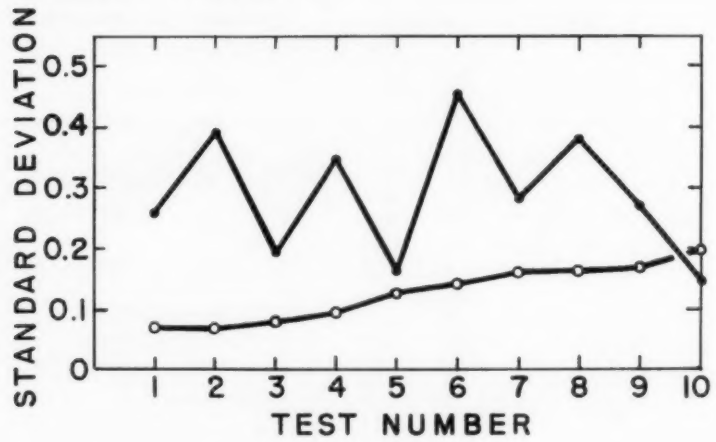

FIG. 1

Comparison of reproducibility among the tests, arranged in increasing order of magnitude for the new method. O——O New Method ●——● Folin Method

7. Rapid Simple Technique and Ordinary Apparatus: In the newer method, the protein-free filtrate is reacted with two stable reagents directly in the calorimeter tube and read in the calorimeter after only a short wait.

Acknowledgments

The authors wish to thank Dr. William H. Parsons, Associate Professor of Physics, Philadelphia College of Pharmacy and Science, for advice in connection with the mathematical treatment of the data, and Dr. W. Eppes, Newark, Delaware, for his help in obtaining serum.

Summary

A new analytical method for the determination of uric acid in serum is described. Advantages include good reproducibility, adherence to Beer's Law over a large range of concentration, low and consistent reagent blanks, stability of reagents and standards, no

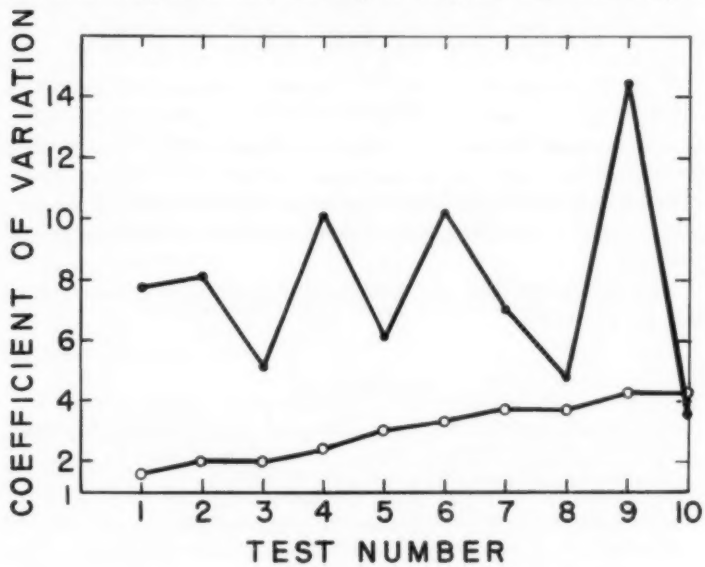

FIG. 2

Comparison of reproducibility of replicates within each test, arranged in increasing order of magnitude for the new method. O——O New Method ●——● Folin Method

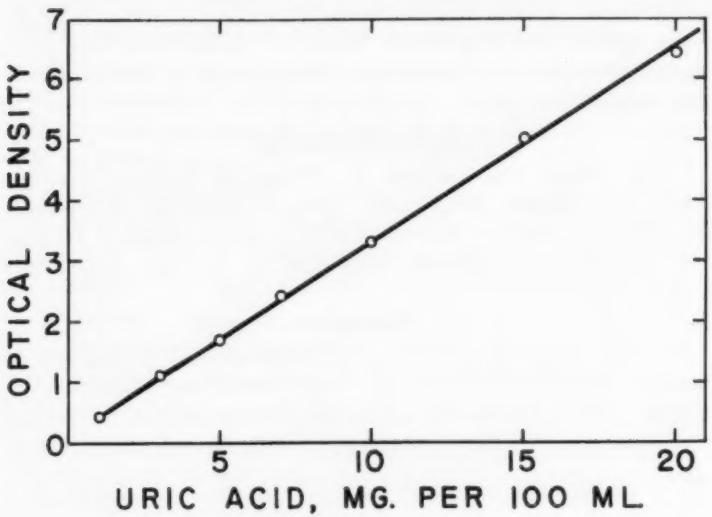

FIG. 3
Uric Acid standard curve.

interference by the formation of cloudiness, the elimination of the very toxic cyanide, a rapid and simple technique, and no need for special apparatus.

Representative data of a comparative study with the method of Folin are presented.

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CURRENT CONCEPTS OF THE MODE OF ACTION OF SALICYLATES IN RHEUMATIC DISEASES

By Philip Needleman *

SALICYLATES were first reported useful in rheumatic diseases in 1876 by Stricker who employed sodium salicylate in the treatment of rheumatic fever. Since that time, salicylates have become of major importance in the therapy of rheumatic disorders. These agents exhibit the ability to reduce the pain, immobility, swelling, and inflammation of the joints, which characterize rheumatic disorders (1).

With the advent of corticosteroid therapy, attention was diverted from the antirheumatic value of the salicylates. However, interest is gradually returning to this aspect of salicylate activity as a result of reports which indicate little difference in the effectiveness of aspirin or cortisone in the long-term control of rheumatic disorders (2).

Considerable effort has been devoted to attempts to elucidate the mechanism by which salicylates ameliorate rheumatic diseases. Since there is no conclusive evidence in support of any single mechanism of action, a number of theories are currently receiving attention. Several of these theories are considered in this brief review.

Pituitary-Adrenocortical Stimulation

Supporting Evidence

A predominating theory considers that the antirheumatic activity of salicylates is attributable to stimulation of the pituitary-adrenocortical system. This hypothesis is largely based on the similarity of effects produced by corticotropin, cortisone, and salicylates in rheumatic disorders. Support was added to this assumption when certain

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rheumatic fever patients, receiving salicylate therapy, evidenced symptoms of adrenal hypersecretion (3).

It has been proposed that salicylates accomplished pituitary-adrenal stimulation by one of the following mechanisms (4, 5). Either: (a) they exert an ACTH-like action, thereby directly stimulating the adrenal cortex to release its steroids; or (b) they stimulate the anterior pituitary, thereby increasing the secretion of ACTH; or (c) they mimic the action of adrenal corticosteroids; or (d) they reinforce their actions in increasing the tissue uptake, blocking the destruction, or increasing tissue sensitivity to the steroids.

Van Cauwenberg (6) administered ACTH to rats and noted a depletion of adrenal ascorbic acid and total cholesterol, eosinopenia, a decrease in the lipid content of the adrenal cortex, and an increase of liver glycogen. Thus, it appears that the salicylates elicit many of the biochemical and hematologic changes produced by ACTH.

Adrenal ascorbic acid depletion is used as an indicator of ACTH activity (7). In rats, salicylates deplete adrenal ascorbic acid and cholesterol, and these effects are abolished by hypophysectomy (8). This evidence suggests that salicylates act via the pituitary-adrenocortical axis (4, 5). Feeney *et al.* (9) have shown that several compounds structurally similar to salicylates caused a similar adrenal ascorbic acid depletion.

Large doses of salicylates caused a significant decrease in the number of circulating eosinophils in rats, guinea pigs, and man (6, 8). Salicylate-induced eosinopenia was prevented in rats by hypophysectomy and adrenalectomy.

Done *et al.* (10, 11) reported that large doses of salicylates increased the levels of circulating adrenal cortical hormones both in animals and normal man. These investigators postulated a qualitative relationship between the increased corticosteroid blood levels and the antirheumatic activity of salicylates. However, they obtained inconsistent elevations of corticosteroid levels when small doses of salicylates were used. They reasoned, therefore, that salicylates not only stimulate the anterior pituitary to release ACTH, but also cause an acceleration of the rate of corticosteroid metabolism (10). This hypothesis serves as a possible explanation for the low levels of corticosteroids found in the urine.

Recent investigations revealed that the blood level of corticosteroids increased only in rheumatic patients who responded favorably

to salicylate therapy, and only when very large doses of salicylates were used. Therapeutic doses of salicylates in rheumatic patients caused no elevation of circulating corticosteroids. This observation suggests that if salicylates increase adrenalcortical activity, it is masked by an accelerated metablism of the corticosteroids. If such a situation actually occurs, it has therapeutic significance since there is suggestive evidence that the metabolism of adrenal hormones is impaired in rheumatic patients (10).

The ACTH-like effects of salicylates were completely blocked by pentobarbital anesthesia (5, 7) and direct local anesthesia of the hypothalamic area (12). These findings suggest that salicylates may act at the level of the hypothalamus, which subsequently mediates the release of ACTH from the anterior pituitary.

The fact that adrenergic blocking agents inhibit epinephrine-induced release of ACTH, but do not block salicylate-induced adrenocortical stimulation, suggests that the latter response is not mediated by epinephrine, liberated as a result of chemical (salicylate) stress (7).

Refuting Evidence

If the antirheumatic effect of salicylates is mediated via the pituitary-adrenal system, then salicylates should uniformly exhibit effects similar to cortisone. However, this is not always the case, particularly with respect to their effects on carbohydrate metabolism (13). Salicylates decrease, whereas cortisone increases, glycosuria in diabetic rats. Contrasting effects also occur in the liver glycogen levels of adrenalectomized rats. Furthermore, rats maintained on high carbohydrate diets and treated with cortisone, developed glycosuria and hyperglycemia, whereas salicylates reduced these effects of cortisone. Thus, salicylates and cortisone not only have opposite actions in regard to carbohydrate metabolism but, in some respects, their effects are antagonistic (4).

No correlation can be drawn between the effects of various salicylate isomers on adrenal cortical function and their antirheumatic properties. Both meta-hydroxybenzoates and para-hydroxybenzoates cause adrenal ascorbic acid depletion in rats, but are devoid of antirheumatic activity (8).

Salicylates have been shown to exert anti-inflammatory effects in hypophysectomized and adrenalectomized animals, apparently eliminating the relationship of these glands to this aspect of salicylate activity (5). It was also noted that salicylates exhibited anti-inflammatory activity during suppressed adrenal function, and that salicylates remained effective antirheumatic agents in patients with Addison's disease (8).

Some workers claim that salicylates, given in therapeutic doses, have no effect on the blood level of corticosteroids in normal patients, acute rheumatic fever patients, or patients with rheumatoid arthritis (8). Other investigators have failed to confirm the finding that salicylates increase the rate of metabolism of corticosteroids, or increase the rate of steroid synthesis, thereby injecting doubt that salicylates reinforce the actions of natural hormones, either by inhibiting their metabolism or by increasing their tissue uptake (5).

Summarization

Those that oppose the hypothesis that salicylates may produce pituitary-adrenal stimulation base their arguments primarily on the differences in action between salicylates and cortisone. Winters and Morrell (14) maintain that large doses of salicylates cause an increase in adrenal corticosteroid output; however, the effects of these steroids on carbohydrate metabolism are conditioned by the presence of salicylates, thereby altering the normal pattern of response. In addition, it is feasible that the antirheumatic effects of these agents may be independent of the effects on carbohydrate metabolism.

Some investigators oppose the pituitary-adrenocortical mechanism theory because of the lack of correlation of structurally related salicylate analogs to antirheumatic action. However, one must keep in mind that structure-activity relationships are only an aid in predicting pharmacologic actions, and that they are frequently evolved by hindsight, rather than foresight. It is not uncommon for exceptions to structure-activity relationships to occur.

Certain critical factors remain unresolved, particularly the effectiveness of salicylates in hypophysectomized and adrenalectomized animals. The bulk of experimental evidence supports the hypothesis of salicylate stimulation of the anterior pituitary-adrenal cortical system, although other mechanisms may also be involved.

Effects on Enzyme Systems

The action of salicylates on certain enzymes may be correlated with their antirheumatic activity.

Hyaluronidase

In 1946, salicylates were reported to inhibit the enzyme hyaluronidase (15). Considerable interest has been focused on this finding for several reasons (16). Hyaluronic acid is an important component of connective tissue, the site of rheumatic disease. Chemically, hyaluronic acid is a mucopolysaccharide composed of glucuronic acid and N-acetylglucosamine in repeating units. It serves as the cement substance in the mesenchyma imparting a viscous barrier and regulating the exchange of water and metabolites in some connective tissue. In the joints, it acts to protect internal surfaces (4).

The enzyme hyaluronidase breaks the bond between the glucuronic acid and N-acetylglucosamine units of hyaluronic acid and causes a reduction in viscosity (17). Hyaluronidase activity is measured by means of the spreading reaction, i.e., intradermal injection of such substances as trypan blue, hemoglobin, or India ink, which allows visualization of the spreading phenomenon (18).

The antirheumatic effect of salicylates is explained on the basis of hyaluronidase inhibition, since an increased activity of the enzyme was reported in rheumatic diseases (4). In this study, salicylates did not exhibit *in vitro* inhibition of hyaluronidase activity. Hemolytic streptococci, which are considered by some investigators to be implicated in the etiology of rheumatic disorders, elaborate hyaluronidase. It was demonstrated that rheumatic patients had abnormal and rapid spreading reactions to hyaluronidase, suggesting removal of the protective viscous layer. Salicylates have been reported to inhibit these enzymatic spreading reactions (16).

Robinson (19), in his review, stated that hypophysectomy and adrenalectomy does not alter the hyaluronidase spreading reaction in rats. In contrast, Pelloja (20) showed that ACTH and cortisone inhibited the spreading action of hyaluronidase in the rat dermis, and that salicylate inhibition of hyaluronidase was abolished after adrenalectomy and hypophysectomy. Therefore, he concluded that salicylates depend upon intact and functional pituitary and adrenal glands for their effects. This conclusion was confirmed by the experiments of Bostrom and Mansson (21), who showed a parallelism between

the effects of cortisone and salicylates on mucopolysaccharides in mesenchymal tissue.

Although there are certain inconsistencies in the data relating the antirheumatic action of salicylates to hyaluronidase inhibition, it is probable that this mechanism plays some role.

Fibrinolysin

Precursors of the enzyme fibrinolysin are believed to play a role in the development of inflammation. Ungar, *et al.* (22) reported inhibition of fibrinolysin *in vitro* using salicylates of known antirheumatic action and other known antirheumatic agents. They concluded that there was a correlation between *in vitro* fibrinolysin inhibition and *in vivo* anti-inflammatory action. Nevertheless, more experimental work must be done with this system before the precise relationship of the enzyme and enzyme inhibitors in the inflammatory process can be established.

Effect of Salicylates in Immunological Processes

The fact that salicylates have been shown to inhibit anaphylactic reactions raised the question of the possible relationship of this phenomenon and that mechanism of salicylate action in rheumatic fever. Done (23), using doses of salicylate comparable to those which provide protection against anaphylaxis, detected elevated blood corticoid levels in animals, and correlated the inhibition of anaphylactic shock with the pituitary-adrenal stimulation by salicylates.

Anaphylaxis was prevented in rabbits by administering aspirin one hour before the sensitized animal was challenged with antigen, thereby suggesting that salicylates affect the immune mechanism by interfering with the interaction between antigen and antibody (24). Salicylates were also shown to inhibit the production of coronary arteritis in sensitized rabbits injected with bovine gamma globulin. Similar effects were obtained with cortisone, thus strengthening the possibility of pituitary-adrenal stimulation (23). Friend (25) observed that sodium salicylate and structurally related compounds inhibited the precipitation of both the antiprotein and anti-polysaccharide antigen-antibody system. The salicylate was found to be an active inhibitor even after the precipitation of antigen and antibody had occurred. This led to the conclusion that salicylates may act by increasing the solubility of the antigen-antibody complex.

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A REVIEW OF THE SUSCEPTIBILITY OF ACETYLSALICYLIC ACID TO DECOMPOSITION

By Edward Stempel *

Historical Background

BATTERMAN (1), quoting other sources, points out that acetylsalicylic acid (hereafter referred to in this paper as ASA) was synthesized by Gerhardt in 1853; however, Hoffman, a chemist at Bayer's chemical works in Germany, found that the substance was successful in therapeutics. Hoffman used ASA for his father's rheumatoid arthritis and influenced Dreser, the director of Bayer's pharmacologic research, to initiate studies. The result was that Dreser presented the first pharmacologic data in 1899 and Wohlgemut presented the first chemical data.

The German company of Bayer introduced ASA under the proprietary name "Aspirin" (2), and established captive production of ASA before the expiration of its Aspirin patent on February 27, 1917 (3). The Monsanto Chemical Company was the first independent producer of the drug in the United States in April 1917 and is considered the world's largest producer of ASA (3). In November 1956, the company produced its 100 millionth pound of ASA (3, 4). In 1956, as in former years, ASA was the medicinal produced in largest quantity; the output was 16.6 million pounds (5).

Obviously, it is desirable to know the stability of the substance that is in such demand.

Stability in the Dry State

The USP (6) states that ASA is stable in dry air, but it gradually hydrolyzes to salicylic and acetic acids in moist air. Leeson and Mattocks (7) investigated the decomposition of ASA in the solid state and found that samples of ASA showed little or no decomposition at 80° or below in the absence of moisture; however, in samples stored at 100° and 110°, salicylic acid was found to increase rapidly

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to about 2 per cent and then decrease gradually with time. When ASA is mixed with other powders in the dry form, it is so sensitive to hydrolysis that it is slowly decomposed by the water of crystallization in the other powders (8).

Decomposition in Solution

The USP (6) states that one gram of ASA dissolves in about 300 ml. of water or 5 ml. of alcohol. Although the USP does not state that decomposition occurs in an aqueous or alcoholic medium, it has been pointed out that ASA hydrolyzes in those solutions, and the rate of decomposition increases with temperature, acids, and alkalies (9).

Ruddiman (10) called attention to the speed with which ASA hydrolyzes by stating that the color produced with the use of a solution of ammonium ferric alum in the presence of the hydrolytic product (salicylic acid) should be observed at once because the (intensity of) color increases decidedly in a few minutes.

Friedlander and Feinberg (11) stated, ". . . ASA should never be used in aqueous solution because of its instability." In addition, Blaug and Wesolowski (12) stated that ASA in aqueous media will hydrolyze almost completely in less than one week.

Levy and Jones (13) have pointed out that organic liquids like alcohol, propylene glycol, and glycerin have been used to prepare solutions of adequate concentration; however, some physicians object to the use of organic solvent vehicles for medication intended for either children or prolonged use. These investigators (13) also stated that such solvents result in a burning or bitter taste, and Blaug and Wesolowski (14) likewise stated that most patients will not find the finished preparation palatable.

Schwarz *et al.* (15) reported that either polyethylene glycol 400 or propylene glycol can be combined with a solution of alcohol with water for the extemporaneous formulation of reasonably stable ASA solutions. Dale (16) states that the freshly prepared Aspirin Elixir of Schwarz *et al.* (17) (containing 2.5% ASA, 20% alcohol, 15% purified water, and sufficient polyethylene glycol 400 to prepare the desired finished volume) should be satisfactory for dispensing. The rate of hydrolysis of ASA in that preparation was about 0.7 per cent per day or about 21 per cent per 5 weeks at 27°, but only 6 per cent per 5 weeks at 6° (15).

Decomposition in pH Adjusted Solutions

Edwards (18, 18a), investigating the aqueous hydrolysis of ASA at 17°, indicated that ASA is most stable at a pH close to 2.5 and saturated aqueous solutions of this substance assume a pH in this region. Garrett (18b) showed the solubilities and extent of hydrolysis of saturated aqueous solutions of various acyl esters of salicylic acid at 25°. He found that the solubility of ASA increases with increased pH and, with increased solubility at a higher pH, there is a greater extent of hydrolysis: at pH 2.25, the solubility of ASA in Gm./L. was 3.41 and the hydrolysis of the saturated solution in Gm./L./Day was 0.153; while at pH 7, the solubility in Gm./L. was 8220 and the hydrolysis of the saturated solution in Gm./L./Day was 2640. Levy and Jones (19) (as well as others subsequently mentioned) pointed out that the use of alkaline substances increases the solubility of ASA and concomitantly results in a preparation in which ASA is rapidly hydrolyzed. Stated in another way (19), the addition of alkaline substances will increase the pH, and this pH together with the greater concentration of drug in solution results in rapid hydrolysis.

The USP (6) states that ASA dissolves with decomposition in solutions of alkali hydroxides or carbonates. Wilson and Gisvold (20), citing Tomski and Waller, state that aqueous solubility of ASA may be increased by using acetates or citrates of alkali metals, although these are said to slowly decompose ASA. In 1960, Bolton (21) stated that citrate salts have enjoyed popularity as ASA "solubilizers" despite the reports that such solutions are relatively unstable.

Morton (22) in 1933 cited the works of Leech, Ruddiman, Stroud, Wilson, and Dott, who showed that aqueous solutions of ASA in the presence of alkali-metal citrates or acetates or bicarbonates will undergo hydrolysis during storage.

Leech (23) indicated that publications appeared that claimed that ASA may be dispensed in solution by the aid of sodium citrate without dissociation. However, he pointed out that such claims were probably due to misinterpretation of the results with the use of ferric chloride solution to detect the presence of free salicylic acid; the misinterpretation was due to the interference of the sensitivity of the reaction by the use of citric acid or citrates to facilitate the solubility of ASA. Ruddiman (24) also pointed out that the statement has been made in pharmaceutical journals that a permanent solution of ASA can be made by dissolving it in a solution of alkali citrate. He ex-

plained that the citrate aids solution possibly by the formation of the alkali acetylsalicylate and citric acid, but the citrate does not prevent the hydrolysis of the acetylsalicylate. Similar to the statement by Leech, Ruddiman (24) said that the violet color with a ferric solution may not show at first because citric acid and citrates interfere, but the color will develop after the solution remains for a time.

Leech (25) found that ASA hydrolyzed fairly rapidly in sodium citrate solution: 50 per cent decomposed in 4 days, 75 per cent decomposed in 9 days, and almost completely hydrolyzed in 17 days. He therefore said that a patient taking the 9-day old combination would be getting essentially the same ingredients as if sodium acetate and sodium salicylate had been used in place of the ASA. In addition, Leech assumed that the suggestion to use one part of potassium citrate instead of 4 parts of sodium citrate to facilitate the aqueous solubility of ASA was incorrect because he assumed that the ASA would hydrolyze faster than in a solution made with a higher concentration of the sodium salt.

Morton (26), investigating the rate of decomposition of ASA in aqueous solutions of sodium citrate as compared to aqueous solutions of potassium citrate, at room temperature without thermostatic control, found that the percentage rate of decomposition is independent of the absolute and relative concentrations of the ASA and the alkali-metal salt and increases very rapidly with a rise in temperature. Regardless of whether sodium citrate or potassium citrate was used to facilitate the aqueous solubility of ASA, the ASA in such solutions decomposed to the same extent—over 10 per cent during the first day or approximately 50 per cent in the course of one week (27).

Stroud (28) showed detailed experiments indicating that ASA is apparently soluble in solutions of other saline salts; however, hydrolysis takes place in each case and the rate increases considerably with a rise in the temperature of the solution. Stroud (29) later demonstrated that ASA decomposition proceeded at the same rate in solutions of either potassium acetate or citrate.

Dott (30) showed that an aqueous solution containing about 3 per cent ASA and 2.8 per cent sodium bicarbonate will have about 96 per cent of the ASA content remaining after 4 hours and about 86 per cent remaining after 24 hours. Therefore, he considered the decomposition to be gradual with negligible changes within a short time. Furthermore, he later showed that it is evidently a matter of

indifference whether sodium bicarbonate or potassium citrate is used to effect solution because the velocity of decomposition is practically the same: about 81.6 percent of the ASA content remains after 18 hours and about 69 per cent remains after 3 days (31).

In apparent contradiction to the fact that aqueous citrate solutions of ASA are unstable, Bowey suggested in 1957 that such solutions are stabilized as compared to solutions containing phosphate, acetate, or calcium hydroxide (21). Bowey (32) tested various buffers and solubilizers (such as sodium acetate, sodium acetate and acetic acid, potassium citrate, potassium citrate and citric acid, sodium phosphate, or ammonium chloride) and reported the most stable formula to be a solution containing 4.4 per cent ASA solubilized by 8.8 per cent potassium citrate. The ASA in that formula hydrolyzed 25 per cent in one week at room temperature.

Schwarz (33) stated in 1958 that the addition of potassium citrate to a liquid ASA preparation is unsound because the ASA rapidly hydrolyzes at the pH of a potassium citrate solution. In addition, Bolton (34) stated in 1960 that, since a citrate complex cannot be demonstrated at low concentrations of citrate, it is difficult to rationalize previous reports (like the one by Bowey) of increased stability of ASA in aqueous citrate solutions. Bolton (35) measured the hydrolytic decomposition of 0.055 molar ASA (about 1 per cent ASA) in aqueous solutions at 30° in the presence of such additives as sodium acetate, citrate, hydroxide, phosphate, or citrate with phosphate. In varying the concentration of the additive as well as the pH in an effort to determine the effect of such changes on ASA stability, Bolton (36) found that, with the exception of greater degradation in the citrate with phosphate solution, the rate of decomposition was about the same regardless of the concentration and nature of the additive at a specific pH. Furthermore, Bolton (37) found essentially no difference in the rate of decomposition of ASA in an aqueous solution containing a larger amount or therapeutic concentration of ASA (0.222 molar ASA or about 4 per cent ASA) with sodium citrate and potassium citrate as compared to other solutions of corresponding pH used in the study. However, he also found that the rate of hydrolysis of 0.055 molar ASA was slightly higher at a pH above 6 than in 0.055 molar ASA solutions of lower pH (36).

Clark (38) found that the presence of sucrose in a potassium citrate solution of ASA resulted in a slower rate of decomposition.

He showed that during 30 days there was more hydrolysis of ASA in an aqueous solution of potassium citrate than in a saturated sucrose solution of the same combination; furthermore, the less the sucrose content the more the hydrolysis.

In the light of Bolton's recent publication (35), it must be emphasized that the pH of the ASA solution is the major consideration in determining the rate of hydrolysis.

ASA in Suspensions

James (39) has reported that the hydrolysis of ASA at room temperature in a *British Pharmaceutical Codex* preparation containing 3.34 per cent ASA suspended with 2.29 per cent compound tragacanth powder in chloroform water was as follows: 1-4 per cent after one week, 3-5 per cent after 2 weeks, 5-6 per cent after 3 weeks, and about 11 per cent after 47 days. Blaug and Wesolowski (14) have stated that suspensions of ASA show a low degree of hydrolysis relative to the total amount of drug in the suspension (as compared to the total amount of drug in solution).

Temperature has been shown to have a marked effect on the rate of hydrolysis of ASA in a suspension: hydrolysis increased 1.5-2 times with a temperature rise from 20° to 34° (40). Therefore, it is important that ASA suspensions be kept in a cool place.

James (41) explained the rate of hydrolysis in ASA suspensions to be independent of the quantity of ASA in the suspension, and suggested that the (hydrolytic or decomposition) reaction is in two parts: (a) solution of ASA followed by (b) decomposition of the ASA in solution. Furthermore, James (42) explained that, as the ASA in solution decomposes, more dissolves, so that the rate of hydrolysis depends on the strength of the saturated solution. Levy and Jones (43) have likewise pointed out that the hydrolysis rate in a suspension is dependent on the amount of drug in solution and not on the total drug concentration. Although ASA hydrolysis is independent of total drug concentration in either a solution (26, 35) or a suspension (41, 43), there is a mathematical relationship between ASA hydrolysis and total drug concentration in a suspension. James (40) found that the rate of ASA hydrolysis (in a suspension) is inversely proportional to (total drug) concentration, and a suspension containing about four times the quantity of ASA as compared to a 3.43 per cent concentration will hydrolyze only about one-quarter

as much in a given time. Levy and Jones (43) likewise stated that, if a 6 per cent ASA suspension shows 6.7 per cent hydrolysis in 31 days at 25°, then a 12 per cent suspension would show only about 3.35 per cent hydrolysis. Blaug and Wesolowski (44) have likewise stated that the per cent hydrolysis of ASA based on total ASA concentration would be smaller for more concentrated suspensions.

Levy and Jones (45) have developed a formula for a palatable ASA suspension of good stability prepared from a powder mixture by the addition of water. The powder mixture consists of: 6 Gm. ASA, 1 Gm. raspberry imitation flavor, 1 Gm. hydroxyethylcellulose, 70 Gm. crystalline d-sorbitol, 0.1 Gm. citric acid, 0.12 Gm. methylparaben, and 0.02 Gm. propylparaben. The suspension must be constituted by adding 56 ml. of purified water in order to obtain 100 ml. of finished suspension containing about 0.3 Gm. of ASA per 5 ml. teaspoonful. The stability of the suspension has been attributed to the sorbitol (43, 14).

Levy and Jones (43) showed that their ASA suspension of relatively good stability did undergo hydrolysis; however, the hydrolysis of ASA was 29 per cent less than in the aqueous controls. They also found that the hydrolysis of their 6 per cent ASA suspension was 6.7 per cent of the total ASA after 31 days at 25° or about 1 per cent after 5 days as compared with a 6 per cent ASA solution at pH 5 wherein 78 per cent is hydrolyzed in 5 days.

Blaug and Wesolowski (44) studied the effect of glycerin in aqueous and buffered suspensions of ASA and found that glycerin increased the hydrolysis rate of ASA. Since the hydrolysis rate is increased with an increased concentration of ASA in solution, the increased hydrolysis rate was probably due to the better solvent action provided by the addition of glycerin (44). Furthermore, these investigators (44) found that calcium gluconate also accelerated the hydrolysis of ASA in a suspension preparation because of the high pH of the aqueous solution. On the other hand, these investigators (46) found that either polyethylene glycol 6000 or polyvinylpyrrolidine exhibited apparently dramatic stabilizing effects on ASA in suspensions. However, when either of these was used, an insoluble gummy sediment formed and was difficult to disperse.

Buckwalter obtained U. S. patent 2,916,416 for coconut oil suspensions of ASA which he claims are highly palatable, acceptable, and stable. Furthermore, he claims that the suspensions are stable at room temperatures for more than 2 years.

In the light of the studies of ASA in suspensions, the inference may be made that the hydrolysis rate in suspensions is dependent on the amount of ASA that is soluble, and (as in aqueous solutions) an additive that contributes alkalinity (to the solution of ASA in the suspension) may accelerate the hydrolysis of ASA.

Conclusion

ASA is stable in the dry state up to 80° and, in consequence of the relatively rapid hydrolysis in solution, the use of suspensions is preferred even though most of these are also known to decompose.

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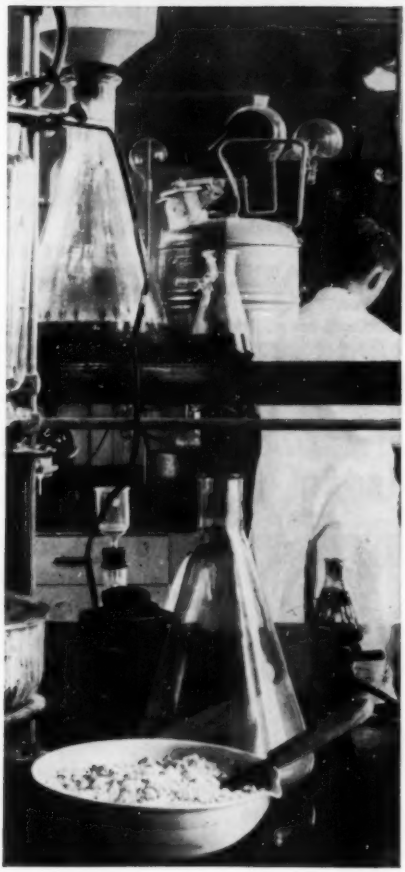

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